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Original Article

Identification of *Trichosporon* yeast isolates from superficial infections in male patients from Central Brazil: an approach to the diversity of infections caused by this basidiomycete fungus

Identificação de isolados de leveduras do gênero *Trichosporon* de infecções superficiais em pacientes do sexo masculino do Brasil Central: uma abordagem sobre a diversidade de infecções causadas por esse fungo basidiomiceto

D.P. Leite Júnior[a,b,c,d*], E. C. de Oliveira[a,b], K. R. Vasconcelos[a], V. K. Vivi-Oliveira[a], M. L. da S. Maia[e], G. M. Oliboni[c], M. B. Macioni[c], I. D. de Oliveira[f,g], J. P. F. Takahashi[f,h], L. X. Bonfietti[d] and M. de S. C. Melhem[c,h,i,j]

[a]Laboratório Central de Saúde Pública do Estado de Mato Grosso – LACEN/MT, Cuiabá, MT, Brasil.
[b]Universidade Federal do Estado de Mato Grosso (UFMT), Laboratório de Pesquisa, Faculdade de Medicina, Cuiabá, MT, Brasil.
[c]Secretaria de Saúde, PPG em Ciências, Coordenação de Controle de Doenças (PPG-CCD-SES), São Paulo, SP, Brasil.
[d]Instituto Adolfo Lutz, Laboratório de Parasitologia e Micologia, Unidade de Micologia, São Paulo, SP, Brasil
[e]Universidade de São Paulo, Instituto de Ciências Biomédicas, São Paulo, SP, Brasil.
[f]Instituto Adolfo Lutz, Laboratório de Patologia, São Paulo, SP, Brasil.
[g]Instituto de Assistência Médica aos Servidores Públicos do Estado de São Paulo, PPG em Ciências da Saúde, São Paulo, SP, Brasil.
[h]Universidade Federal de Mato Grosso do Sul (UFMS), Faculdade de Medicina, PPG em Parasitologia e Doenças Infecciosas, Campo Grande, MS, Brasil.
[i]Universidade do Estado de São Paulo, Faculdade de Medicina, PPG em Doenças Tropicais, São Paulo, SP, Brasil
[j]Instituto de Medicina Tropical, São Paulo, SP, Brasil

Abstract

The genus *Trichosporon* are currently recognized as opportunistic pathogens capable of causing superficial “white piedra” infections and potentially fatal invasive diseases (Trichosporonosis). In this work, determine the agent *Trichosporon* spp. isolated from the skin and appendages of a male population group in the Central-West region of Brazil. The isolates were analyzed by phenotypic, biochemical and molecular methods. Twenty-five strains of *Trichosporon* were isolated: *T. asahii* (18; 72%), followed by *T. inkin* (4; 16%) and *T. faecale* (3; 12%). Skin infections were the most affected (16; 64%) and the genitocrural region (13; 52%) was the most affected. The highest rate of isolation occurred between the ages of 21 and 30 years (9; 36%), with black men (African descent) (13; 52%) being the most affected by this type of superficial infection. After the advent of molecular techniques, more than 50 subspecies and about 16 different strains have been reported to cause human disease. In this series, three species of the genus *Trichosporon* of medical importance were highlighted, colonizing the genital and perigenital region of the studied population. For the identifications, classical phenotypic methods associated with genotypic identification were carried out, using molecular techniques based on the study of DNA; using sequence analysis of the DNA intergenic spacer region 1 (IGS1).

Keywords: Trichosporonosis, fungal infection, arthrosporate yeast, arthrospore, piedras.

Resumo

O gênero *Trichosporon* é atualmente reconhecido como patógeno oportunista capaz de causar infecções superficiais por “piedra branca” e doenças invasivas potencialmente fatais (Tricosporonose). Neste trabalho foi determinado o agente *Trichosporon* spp. isolados da pele e anexos de um grupo populacional masculino na região Centro-Oeste do Brasil. Os isolados foram analisados por métodos fenotípicos, bioquímicos e moleculares. Foram isoladas 25 cepas de *Trichosporon*: *T. asahii* (18; 72%), seguida de *T. inkin* (4; 16%) e *T. faecale* (3; 12%). As infecções de pele foram as mais acometidas (16; 64%) e a região genitocrural (13; 52%) foi a mais acometida. A maior taxa de isolamento ocorreu entre as idades de 21 e 30 anos (9; 36%), sendo os homens negros (afrodescendentes) (13; 52%) os mais acometidos por esse tipo de infecção superficial. Após o advento das técnicas moleculares, mais de 50 subespécies e cerca de 16 cepas diferentes foram relatadas como causadoras de doenças humanas. Nesta série foram destacadas três espécies do gênero *Trichosporon* de importância médica, colonizando a região genital e perigenital da população estudada. Para as identificações foram realizados métodos fenotípicos clássicos associados à identificação genotípica, utilizando técnicas moleculares baseadas no estudo do DNA; usando análise de sequência da região espaçadora intergênica 1 do DNA (IGS1).

Palavras-chaves: Trichosporonoses, infecções fúngicas, leveduras artroporadas, artroporos, piedras.

*e-mail: djbiologico@gmail.com
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1. Introduction

Some fungi are natural inhabitants of the human body, but can result in diseases when conditions are conducive to their development (Leite Júnior et al., 2011). Disease spread by fungi are a One Health issue, fungi that cause human diseases live in the environment and some can spread between animals and people. Fungal infections that affect the skin include dermatophytes, yeasts of the genus Candida and other species of basidiomycetic yeasts, such as those of the genus *Trichosporon*, which can be found ubiquitously in nature and which, together or separately, can promote superficial and systemic infections, depending on the host's immune status, often with involvement of organs other than the skin.

The genus *Trichosporon* are anamorphic, yeast-like organisms, Basidiomycetes (*Basidiomycota, Tremellomycetes, Tremellales, Trichosporonales, Trichosporonaceae*), without sexual phase widely known and distributed in nature and around the world, occurring in all three subphyla of Basidiomycota, *Agaricomycotina, Pucciniomycotina* and *Ustilaginomycotina,* currently recognized based on molecular phylogenetic analyses (Liu et al., 2015a, b).

The word *Trichosporon* originates from the Greek vocabulary Trichos (hair) and sporon (spores) and was first discovered by Biegel in 1865, who observed that this organism caused benign capillary colonization (Kourti and Roilides, 2022). The genus *Trichosporon* is characterized by the production of septate hyphae, arthroconidia, yeasts and pseudohyphae and by yeast-like growth in culture media (Bennet et al., 2015).

The genome sketch of the order Trichosporonales currently consists of two families; *Trichosporonaceae* and *Tetragoniomycetaceae*, the first includes six genera (*Apiotrichum, Cutaneotrichosporon, Effuseotrichosporon, Haglerozyma, Trichosporon* and *Vanrija*) and the second order includes four fungal genera (*Bandonia, Cryptotrichosporon, Takshimella* and *Tetragoniomyces* (Liu et al., 2015a, b). Takashima (Takashima et al., 2019) in a new phylogenetic analysis, suggested including the genera Pascua and Prillingera in the family Trichosporonaceae.

Several species occur as a natural part of the skin, respiratory, and digestive tract microbiota of 2 to 5% of humans and are found in animals (Kurtzman et al., 2011). In humans, this fungal species is occasionally found as a member of the superficial, gastrointestinal and oral cavity microbiota, transiently colonizing the respiratory tract, skin, perianal region, and male and female genital organs.

The genus is often a causative agent of skin infections known as white piedra, a harmless infection of the hair shaft found mainly in tropical and temperate countries and worldwide distribution, including Europe, Asia, Japan and the southern United States (Chander, 2018) and in Latin America, including the Brazil.

The most common clinical presentation of superficial *Trichosporon* infection is benign irregular nodules on the hair shaft lesions, called white Piedra (Chander, 2018). The nodules are soft in texture and are loosely attached to the hair shaft, varying in hue from white to light brown. Due to the proximity to the genital regions where fungal infections are more common, they can facilitate the spread of the infection towards the perineal, perianal and inguinal regions (Duldulao et al., 2019). There may be a relationship between the basidiomycetic yeast and bacteria of the genus *Corynebacterium* simultaneously infecting the genital hair shafts (Kalter et al., 1986).

Trichosporonosis is a type of fungal infection that mainly affects immunosuppressed patients, with a difficult prognosis and generally with a high mortality rate. These infections have increased in recent years due to factors such as the incidence of malignancies and exposure of patients to immunosuppressive treatments, chemotherapy, broad-spectrum antibiotics, invasive procedures, and organ transplantation (Chagas-neto et al., 2009; Espirito-Santo et al., 2020).

Yeasts of the genus *Trichosporon* have specialized mannose structures that inhibit the action of phagocytosis by the host's macrophages. Its composition produces a biofilm that facilitates the colonization of internal devices, allowing adhesion to hospital, prosthetic, biotic, and abiotic materials and protection against antifungal drugs (Di-Bonaventura et al., 2006). Its high capacity for morbidity and mortality can be attributed to multiple virulence factors presented by this fungus, among them the production of biofilm on several surfaces and its exopolymer matrix that constitutes a biofilm that presents a physical-chemical barrier giving the fungus greater tolerance to antifungal agents, hindering the penetration of these drugs and, consequently, their action on the pathogen (Colombo et al., 2011).

The genus *Trichosporon* is one of the oldest fungal genera, the reclassification of the species *T. beigelii* (Küchenm. & Rabenh.) Vuill., 1992, which is currently called *T. asahii* (Akagi ex Sugita, A. Nishikawa & Shinoda, 1994) (Guého et al., 1992). About 52 species are widely present in nature, 16 species have been identified as human pathogens of which they are considered of medical interest (Colombo et al., 2011) and some have pathogenic potential, as they are important opportunistic pathogens and also cause infections in humans, such as *T. asahii, T. inkin* (Oho ex M. Ota) do Carmo-Sousa & van Uden, 1967), *T. asteroides* (Rischin) M. Ota, 1926) and *Cutaneotrichosporon mucoides* (E. Guého & M.T. Sm.) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, 2015), responsible for most cases of Trichosporonosis.

The genus *Trichosporon* is undergoing new taxonomic revisions; proposed new genera, in addition to the species already incriminated as pathogens, new proposals in the genera *Trichosporon, Cutaneotrichosporon, Apiotrichum, Effuseotrichosporon, Haglerozyma* and *Vanrija,* were recorded for the pathogenic species within *Trichosporon*aceae family (Liu et al., 2015a, b).

The new classifications are based on phenotypes that are largely in conflict with molecular phylogenetic analyses, which are being recognized and evaluated through sequence data. Studies have shown that co-specific strains have less than 1% nucleotide differences in ITS regions and these proposals meet the need to improve species identification (Liu et al., 2015a, b; Guo et al., 2011).

Four species *Trichosporon asahii, T. asteroides, T. inkin,* and *T. ovoides* (Behrend, 1890) are the most common clinical isolates, however, *T. coremiiforme* (M.

Moore) E. Guho & M.T. Sm., 1992), *T. dohaense* (Taj-Aldeen, Meis & Boekhout), *T. faecale* (Bat. & J.S. Silveira) E. Guho & M.T. Sm., 1992), *T. japonicum* (Sugita & Nakase, 1998) and *T. lactis* (Lopandic, Sugita, Middelhoven, Herzberg & Prillinger, 2004) have also been reported from human and animal infections (Guo et al., 2019; Rodriguez-Tudela et al., 2005; Guo et al., 2011; Chagas-Neto et al., 2009). *Trichosporon* spp. are resistant to Echinocandins and Amphotericin-B resistance is also frequently reported in research for *T. asahii, T. coremiiforme, T. faecale* and *Apiotrichum loubieri* (Morenz) A.M. Yurkov & Boekhout, 2015) (Guo et al., 2019; Marty et al., 2003; Espinel-Ingroff, 2003; Paphitou et al., 2002). An increase in the resistance of *Trichosporon* spp. to antifungals are being commonly reported in the literature (Almeida Júnior et al., 2021), the evaluation of the in vitro susceptibility of biofilms has been tested with the antifungals currently used (amphotericin B, caspofungin, fluconazole and voriconazole) (Lara et al., 2023; Di-Bonaventura et al., 2006).

Triazoles show greater activity against *Trichosporon* species, especially the new generation antifungals, voriconazole, posaconazole, ravuconazole and isavuconazole (Rodriguez-Tudela et al., 2005; Marty et al., 2003; Espinel-Ingroff, 2003; Paphitou et al., 2002). The use of antifungals fluconazole and voriconazole are recognized as alternatives for the treatment of invasive and systemic Trichosporonosis (Francisco et al., 2019; Li et al., 2017; Pfaller et al., 2009), even with the limited number of drugs available that require antifungal activity against species of the genus *Trichosporon* (Li et al., 2017; Guo et al., 2011).

Molecular techniques based on the study of DNA have been widely used to characterize and identify the genus *Trichosporon* spp., using sequence analysis of the intergenic spacer region 1 of DNA (IGS1), which has been the appropriate tool and provided a molecular diagnosis to differentiate species of this fungal genus (Rodriguez-Tudela et al., 2005; Dabas et al., 2017; Sugita et al., 2002). According to data provided by the epidemiological surveillance of ARTEMIS DISK, *Trichosporon* spp. are considered the second or third most commonly isolated yeast species in clinical laboratories, representing 5.5 to 10.6% of all isolates (Pfaller et al., 2009).

One Health is a currently used approach that recognizes that the health of people is closely connected to the health of animals and our common environment. In this fungal group, its adaptive characteristic to humans and its infectious characteristics are observed. The scalp is the anatomical region most commonly affected by this eukaryotic agent, but this arthrosporous yeast can affect practically all hairy areas of the body, such as armpits, eyebrows, beard, eyelashes, chest, pubis and anal region. Nail infection can also present a variety of distinct signs, such as onychomycosis, paronychia and other clinical manifestations. This fungal agent can also be isolated from the bloodstream and urine, promoting infections depending on the patient's immune status. Having as the etiological agent the arthrosporous yeast *Trichosporon* spp; It is considered relevant to verify the distribution and epidemiological profile of this yeast species, carrying out the phenotypic characterization of this emerging fungal genus.

2. Materials and Methods

2.1. Demographic data

In this study, 55 male patients were evaluated, who reported complaints on the genitals, scalp and nails with symptoms and/or lesions compatible with or suggestive of superficial fungal infections. Patient samples were collected from different anatomical sites of human origin (skin scrapings, perineal, genital, inguinal hair, buttock skin, nails, scalp and beard), in the period from 2016 to 2018. All patients were seen in two hospitals in the city of Cuiabá, Mato Grosso, central region of Brazil, and information regarding demographic data was collected: age, sex, profession, race and education, informed by patients at the time of collection (self-declared), Under the approval of the REC (Research Ethics Committee) protocol no. 495/HUJM/2018. Biological samples were collected according to the anatomical site and the characteristics of the injuries reported by the individuals, skin and nail scraping, cutting and/or shaving of scalp and body hair.

Of the 55 clinical isolates, 25 could be identified, had appearance and lesions compatible with superficial fungal infections and were concomitantly associated with dermatophytic fungi and yeasts of the *Candida* and *Trichosporon* genera, isolated in the same topography. Clinical specimens of yeasts of the genus *Trichosporon* spp., the target of this study, were isolated in Sabourraud agar culture medium and sent and analyzed by the mycology laboratory of Instituto Adolfo Lutz/SP-Brazil, where the strains were cultivated, purified and identified by phenotypic methodology and sequencing.

2.2. The climate of the researched region

In Cuiabá, the Capital of Mato Grosso, Brazil, and Geodetic Center of Latin America, the precipitation season is oppressive and overcast, with well-defined seasons, a dry one (autumn-winter) and a rainy one (spring-summer) (Leite Júnior et al., 2011). During the whole year, the climate is hot, due to its Köppen-Geiger Aw climate characterization, semi-humid sub-tropical. Throughout the year, in general, the temperature varies from 18°C to 35°C and is rarely below 14°C or above 39°C. The hot season lasts from July to October, with a daily average maximum temperature above 34°C. The hottest months of the year in Cuiabá are September and October, with a maximum of 34°C and a minimum of 24°C, on average.

The rainy season starts in December, peaking in January and ending in June, with a daily maximum temperature below 31°C on average. The month that temperature drops can occur in the year is July, with a maximum of 19°C and a minimum of 32°C, on average. To assess the relationship between climate and the occurrence of superficial *Trichosporon* infections or positive cultures, the average temperature in Cuiabá. The data in this report originated from Marechal Rondon International Airport each month and obtained from the Central do website (pt. Weatherspark.com) (Weather Spark, 2023).

2.3. Phenotypic identification

The parameters used for physiological identification and identification standardization were based on previous studies recommended by De Hoog (De Hoog et al., 2007) and Kurtzman and Fell (Kurtzman et al., 2011). Traditionally, phenotypic identifications were performed, observing from a macroscopic point of view, colonies with a cerebriform appearance, with a white to cream color, on Sabourraud dextrose agar medium (Figure 1). In this study, in the microscopic analysis of these cultures, the presence of: arthroconidia, blastoconidia, appressoria formation, pseudo-hypha, true hyphae (Colombo et al., 2011), apical giant cells, and barrel-shaped cells were investigated, observed on cornmeal-tween 80 agar on slides take under an optical microscope. The phenotypic characterization of clinical isolates was performed through micromorphological characteristics (Sidrim and Rocha,

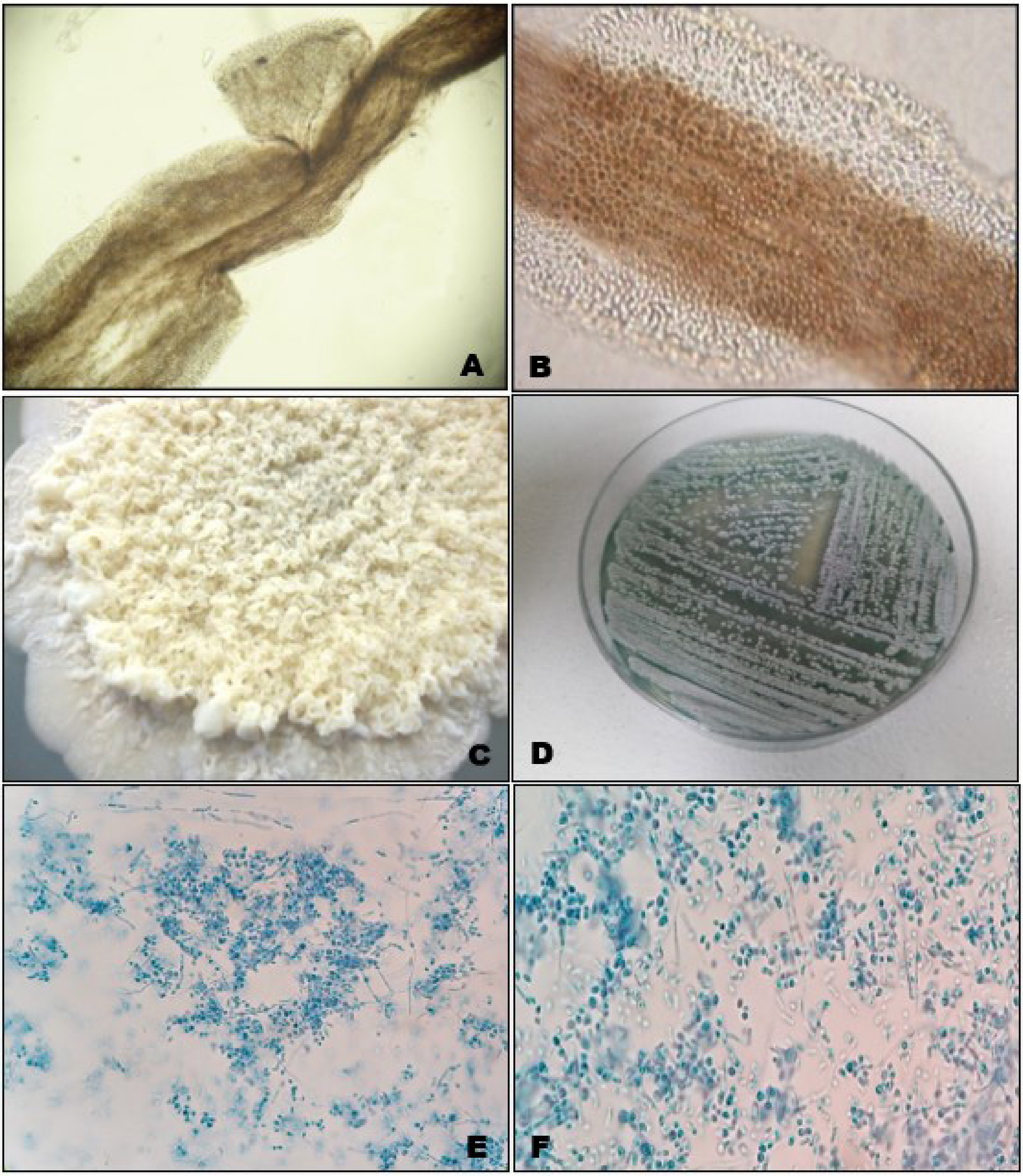

Figure 1. Macroscopic and microscopic characteristics of *Trichosporon asahii.* A-B: Direct examination. Hyaline nodule observed under a microscope showing arthroconidium and some blastoconidium, adhered to the pubic hair. Macroscopic and microscopic characteristics of *Trichosporon asahii*. A-B: Image observed under a microscope showing yeast-like structures adhered to the hair. C: Macromorphological aspect of *T. asahii*; growing on a plate containing sabouraud agar, showing a cerebriform aspect of the colony, with a white to cream color. D: Macromorphological aspect of colony on CHROMagar Candida. E-F: Micromorphological aspect of yeasts. Hyaline septate hypha's and arthroconidia observed with cotton blue stain (lactophenol blue). Image: Leite Júnior, DP.

2004), and this identification was confirmed by molecular studies (genotypic identification).

For genus confirmation, 24-72h repeats, maintained on Sabouraud dextrose agar, were submitted to the urease production test in *Christensen's urea agar medium*, incubated at 37º C for up to 72 hours. All *Trichosporon* species hydrolyze urea, differing from the genus Geotrichum, which also produces arthroconidia (Chander, 2018). Clinical strains of *Cryptococcus neoformans* (ICB 162C) and *Candida albicans* (ICB 12A) were used as positive and negative controls, respectively.

The medium with cycloheximide can help identify since it allows the selection of resistant yeasts to this compound. The 0.1% cycloheximide tolerance test was performed on all yeasts, which were seeded in tubes containing Sabouraud dextrose agar supplemented with 0.1% cycloheximide and incubated at 27ºC for 10-15 days.

Evaluation of purity and viability of samples stored at -70°C were seeded in selective chromogenic medium CHROMagar Candida®, to certify the purity of the culture and viability of the colonies. After 48 h of incubation at 37°C, colonies were selected on Sabouraud dextrose agar plus chloramphenicol.

The phenotypic characteristics currently used do not resolve the characteristics of correct distinction between *Trichosporon* and related genera.

2.4. Biochemical characterization

For the biochemical characterization of the samples, API 20C®AUX (BioMérieux, Marcy l'Etoile, França) commercial kits were used, with observation of the carbohydrate assimilation pattern (glucose, galactose, maltose, sucrose, lactose, trehalose, xylose, melibiose, raffinose and cellobiose) as a carbon source. Other additional physiological tests were also used for the assimilation of carbon and nitrogen sources (glycerol, rhamnose, arabinose, ribitol, adonitol, galactitol, xylitol, inositol, sorbitol, methyl D-glucosamine, acetyl, glucosamine and melezitose) and nitrogen, used as a source of nitrate assimilation.

2.5. Genotypic identification

Reference strains of *T. inkin* (CBS 5585), *T. asahii* (CBS 2479), *T. mucoides* (CBS 7625), *T. asteroides* (CBS 2481) and *T. ovoides* (CBS 7556) were used as controls and benchmarks for phenotypic and molecular studies of species identification for isolates of *Trichosporon* spp.

For genotypic identifications, samples collected from lesions compatible with a fungal infection were identified by molecular analysis of the IGS1 rDNA region after phenotypic and biochemical tests. For extraction, the samples were cultured on Sabouraud dextrose agar medium containing chloramphenicol and incubated at 35-37ºC for 48 h.

Amplification of the IGS1 region was obtained with primers 26SF (IGS1-26SS) (5'-TCCGTAGGTGAACCTGCGG-3') and 5SR (IGS2-58S) (5'-AGCTTGACTTCGCAGATC GG-3') (Dabas et al., 2017; Sugita et al., 2002). Molecular identification was performed by amplification and sequencing of the IGS1 region from the rDNA according to the protocol previously described (Chagas-Neto et al., 2009). Sequencing of 2 to 4 reads per isolate was performed with the BigDye Terminator Kit (Applied Biosystems) protocol, and the final sequence was obtained after alignment and edition in Sequencer TM 4.1.4 - Gene Codes. Final species identification was obtained by comparisons of the sequences of the IGS1 rDNA region of all identified isolates were aligned with sequences from species deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Kumar et al., 2016). They were accepted with maximum identity coverage query ≥99%, identity ≥99% and E value = 0.0.

3. Results

Of the 55 samples initially isolated, 25 (45.4%) could be isolated and identified as yeasts of the genus *Trichosporon*, namely: 18 strains identified as *T. asahii*, 4 strains identified as *T. inkin* and 3 strains identified as *T. faecale*, in the clinical materials skin, hair and nails (Table 1). *Trichosporon asahii* was the most prevalent species found (18, 72%), followed by *T. inkin* (4, 16%) and *T. faecale* (3, 12%) isolated in the researched population. All samples came from superficial infections, being; skin (16; 64%), hair (7; 28%), and nails (2; 8%) of a male population group (Table 1).

The participants involved, whose ages ranged between 18 and 53 years, with a mean = 27.6; SD = 8.6; 95% CI (3.4). The species *T. asahii* was the most frequently isolated in all age groups evaluated; being highlighted in the age group of 31-40 years, and the age group of 21-30 years was the most affected by infections among the three isolated species (Table 2).

Table 1. Number of isolates of species of the genus *Trichosporon* in clinical material of skin and its appendages in the masculine gender.

Superficial mycosis or skin colonization					
Number of isolates/sources					
Species	**Skin**	**Hair**	**Nails**	**Total**	**%**
T. asahii	11	5	2	18	72
T. inkin	4	0	0	4	16
T. faecale	1	2	0	3	12
Total	16	7	2	25	100

Lesions compatible with infections by yeasts of the genus *Trichosporon* were collected from different anatomical sites such as the beard, scalp, genital region, crural region, buttocks, and nails. The crural region was the most predominantly infected by the lesion (13; 52%), followed by the genital region (4; 16%) establishing lesions called involving the skin and its annexes (Table 3).

All samples collected were from male patients with lesions compatible with fungal infection. The evaluated patients self-declared their ethnicity; Black men were most commonly infected (13; 52%) in our clinical findings, followed by brown men (8; 32%) and then Caucasian men (4; 16%). Different professional activities were reported, and the education level of the participants were high school (15; 60%), followed by higher education (6; 24%) and, finally, fundamental level (4; 16%) (Table 4). It was not possible to establish statistically significant associations between the variables (age, sex and ethnicity) studied and fungal infections and their etiological agents isolated in this investigation.

All isolates showed the observed micro-macromorphological characteristics consistent with those of yeasts of the genus *Trichosporon*: hyphae, pseudohyphae, arthroconidia, and blastoconidia. All samples were characterized as urease-positive, able to grow at 37ºC and presented phenotypic aspects distinguishable from other species, such as: white to cream color, cerebriform aspect, and blue-grey to opaque pink-grey color attributed to CHROmagar Candida®, for purification.

Dermatophytic agents associated with White Piedra agents, causing skin infections in the genital region, affected some patients. Of the 25 patients identified with suspected skin, hair and nail infections, nine patients complained of scaling, erythema and local irritation. Concomitant dermatophyte and *Trichosporon* infections were identified in microscopic observations after clarification with KOH and confirmation after isolation in Sabourraud culture medium. Three species of dermatophytes could be identified, associated with the isolated species of *Trichosporon* concomitantly.

The following isolated dermatophytes were: *Trichophyton rubrum* (Castell.) Sabour., 1911) (4; 16%), followed by *Microsporon canis* ((Bodin) Bodin, 1900) (2; 8%) and finally *Epidermophyton floccosum* ((Harz) Langeron & Miloch., 1930) (1; 4%), six of which were isolated from skin samples (24%) and an isolated hair sample (4%). Of the skin samples, 6 isolates were from the inguinal-crural region (6.24%) and a sample of skin scrapings from the penis (1.4%). Regarding dermatophyte isolates, four isolates were identified as *T. rubrum* in association with *T. asahii*; and two *M. canis* isolates; one being in association with *T. faecale* and the other associated with *T. asahii*. The only isolate of *E. floccosum* was found associated with *T. asahii* (Table 4). Concomitant infections were also found among the genus *Trichosporon* with yeasts of the genus *Candida*, in the skin of the inguinal region, isolating *C. albicans* (C.P. Robin) Berkhout, 1923 in [Berkhout (1923)], and in the skin of the scrotal region, isolating *C. parapsilosis* (Ashford) Langeron & Talice, 1928) sensu strictu. The two Candida yeast isolates were associated with *T. asahii* (Table 4), after the respective identifications.

All dermatophyte isolates were seeded on Sabouraud agar, incubated at 35-37°C for 7-10 days, and identified by the lactophenol blue microculture technique. Yeast

Table 2. The age range of the male population distributed by the species of *Trichosporon* isolated from the skin and its appendages, in the masculine gender.

Species	AGES					
	18 -| 20	21 -| 30	31 -| 40	41 -| 50	Total	%
T. asahii	5	5	6	2	18	72
T. inkin	0	3	1	0	4	16
T. faecale	1	1	1	0	3	12
Total	6	9	8	2	25	100

Table 3. *Trichosporon* species identified in the anatomical sites, causing cases of Trichosporonosis in the skin and its annexes, in the masculine gender.

Species	TRICHOSPORONOSIS							
	Folliculitis	Capillary	Genital-crural			Ungueal		
	bear	scalp	genital	crural-region	buttocks	nails	Total	%
T. asahii	2	1	4	8	1	2	18	72
T. inkin	0	0	0	3	1	0	4	16
T. faecale	0	0	0	2	1	0	3	12
Total	2	1	4	13	3	2	25	100

Table 4. Identification of *Trichosporon* + association isolates from a population group by phenotypic method and sequencing of the IGS region.

PATIENTS								Associated other fungi	IDENTIFICATION/ IGS REGION		
Case	Sex	Age/Years	Race/ Skin color	Schooling	Occupation	Clinical topograph	Anatomical site region		Etiology ID. Mol.	NCBI Seq.	% ID
001 A	M	29	White	medium	autonomous	skin	inguinal region	*T. rubrum*	*T. inkin*	EU934804.1	99%
002 B	M	28	Black	medium	soldier	hair	perineal region	-	*T. asahii*	MK737694.1	100%
003 A	M	35	Black	higher	firefighter	skin	inguinal region	*T. rubrum*	*T. asahii*	MK737694.1	99%
004 A	M	19	Brown	medium	soldier	skin	inguinal region	-	*T. asahii*	MK737694.1	100%
005 A	M	41	Brown	medium	seler	skin	buttocks	-	*T. asahii*	MK737694.1	99%
006 A	M	33	Black	higher	policeman	skin	penis	*T. rubrum*	*T. asahii*	MK737694.1	99%
007 C	M	20	White	medium	marketer	nail	right hand index	-	*T. asahii*	MK737694.1	99%
008 B	M	19	Black	fundamental	stockist	hair	inguinal region	*M. canis*	*T. faecale*	KY109952.1	100%
009 A	M	21	Black	medium	soldier	skin	scrotal region	*C. albicans*	*T. asahii*	MK737694.1	100%
010 A	M	19	Brown	medium	autonomous	skin	penis	-	*T. asahii*	MK737694.1	99%
011 A	M	21	Brown	fundamental	firefighter	skin	perineal region	-	*T. inkin*	EU934804.1	100%
012 A	M	36	Brown	higher	professor	skin	buttocks	-	*T. inkin*	EU934804.1	99%
013 B	M	20	Brown	fundamental	seller	hair	inguinal region	-	*T. asahii*	MK737694.1	100%
014 B	M	27	Black	medium	stockist	hair	inguinal region	-	*T. faecale*	KY109952.1	100%
015 A	M	22	Black	medium	stockist	skin	buttocks	-	*T. faecale*	KY109952.1	99%
016 A	M	18	Black	medium	soldier	hair	scrotal region	-	*T. asahii*	MK737694.1	100%
017 B	M	31	Black	higher	professor	hair	beard	-	*T. asahii*	MK737694.1	99%
018 C	M	53	White	medium	marketer	nail	right hand thumb	-	*T. asahii*	MK737694.1	100%
019 A	M	37	White	medium	soldier	skin	inguinal region	*M. canis*	*T. asahii*	MK737694.1	99%
020 A	M	32	Black	higher	soldier	skin	inguinal region	*E. floccosum*	*T. asahii*	MK737694.1	100%
021 A	M	28	Black	higher	waiter	skin	inguinal region	*T. rubrum*	*T. asahii*	MK737694.1	99%
022 B	M	22	Black	medium	policeman	hair	beard	-	*T. asahii*	MK737694.1	100%
023 A	M	24	Black	medium	soldier	skin	perineal region	-	*T. inkin*	EU934804.1	100%
024 A	M	35	Brown	medium	policeman	skin	scalp	-	*T. asahii*	MK737694.1	99%
025 A	M	21	Brown	fundamental	soldier	skin	inguinal region	*C. parapsilosis*	*T. asahii*	MK737694.1	99%

specimens of the genus Candida were isolated on chromogenic medium CHROMagar Candida®, and the yeast microculture technique was used for observation under a microscope.

4. Discussion

Currently, nine species of yeasts of the genus *Trichosporon*, cause disease in humans, were highlighted: *T. asahii*, which is occasionally found in nature and can be recovered as saprobic from soil or organic material (Espirito-Santo et al., 2020; Li et al., 2017) and also recognized as the most common cause of disseminated infection, *T. inkin*, the most common agent of white piedra; *T. asteroides, Cutaneotrichosporon* cutaneum (Beurm., Gougerot & Vaucher bis) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, 2015*), C. mucoides, T. ovoides, Tausonia pullulans* (Lindner) X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout, 2016), *Apiotrichum loubieri* (Morenz) A.M. Yurkov & Boekhout, 2015) and *T. japonicum* (Liu et al., 2015a, b; Silvestre Júnior et al., 2010; Ağirbasli et al., 2008). In addition to the species already mentioned; Taj-Aldeen (Taj-Aldeen et al., 2009) considers the species *Cutaneotrichosporon dermatis* (Sugita, M. Takash., Nakase & Shinoda) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, 2015) and *T. dohaense* as agents of Trichosporonosis.

DNA-based methods have been widely adopted to accurately identify *Trichosporon* species. The identification of *Trichosporon* species is based on sequence analysis of IGS1 regions, which are variable in related species from conserved regions (D1/D2 regions of 28S rDNAa) (Espirito-Santo et al., 2020; Almeida Júnior and Hennequin, 2016; Sugita et al., 2002; Iturrieta-González et al., 2014).

The IGS1 sequences from *Trichosporon* samples were compared with reference sequences in GenBank, allowing accurate classification of fungal species, as this region has a high potential to discriminate related species, compared to other sites such as the ITS region, resulting in in a phylogenetic analysis which revealed that the IGS1 sequences of all *Trichosporon* samples were 100% identical to the reference sequences (Guo et al., 2011).

The results presented in this study are in line with the information provided by other researchers in the world and in Brazil. In 2020, Brazilian researchers (Espirito-Santo et al., 2020) in sequencing the IGS1 rDNA region, identified all 26 clinical isolates *T asahii* (12), *T. inkin* (4), *T. faecale* (3), *C. dermatis* (2), *T. coremiiforme*, *T. japonicum* and *Apiotrichum mycotoxinivorans* (O. Molnr, Schatzm. & Prillinger) A.M. Yurkov & Boekhout, 2015) (1), with 100% expression in their samples. In Kuwait in 2015, Asian researchers (Ahmad et al., 2005) also reported the practicality and percentage of positivity, when they analyzed 29 *Trichosporon* isolates by rDNA gene sequences, *T. asahii* (25) and *T. asteroides* (4) were identified.

In 2014, researchers from São Paulo/Brazil carried out the production of biofilm and antifungal susceptibility to triazoles and amphotericin B from 54 strains of *Trichosporon* spp. isolates obtained from blood, urine, and superficial mycoses samples. *T. asahii* was the most frequently identified species, followed by *T. faecale, T. asteroides, T. inkin, C. dermatis,* and *T. coremiiforme.* All species showed a high capacity for adhesion and biofilm formation, mainly *T. inkin, T. asteroides,* and *T. faecale* (Iturrieta-González et al., 2014).

In 2021, Indian researchers reported in a literature review, that intrinsic resistance to echinocandins, as well as 5-flucytosine, there are variable minimum inhibitory concentrations for amphotericin B and moderate susceptibility to fluconazole, itraconazole, voriconazole and posaconazole, and that the combination of echinocandins with amphotericin B or azoles showed synergistic antifungal effects (Mehta et al., 2021).

More recently, in 2023, other researchers from São Paulo evaluated aspects related to biofilm production and antifungal susceptibility of clinically relevant yeasts of the genus *Trichosporon*, analyzing *T. asahii* (n=31), *Cutaneotrichosporon debeurmannianum* (Sugita, M. Takash., Nakase & Shinoda) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, 2015) (n=11), *T. inkin* (n=8), *C. dermatis* (Sugita, M. Takash., Nakase & Shinoda) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, 2015) (n=3), *T.* coremiiforme (M. Moore) E. Guho & M.T. Sm., 1992 (n=2), *Apiotrichum montevideense* (L.A. Queiroz) A.M. Yurkov & Boekhout, 2015 (n=1), *A. veenhuisii* (Middelhoven, Scorzetti & Fell) A.M. Yurkov & Boekhout, 2015) (n=1), *T. insectorum* (Fuent., S.O. Suh, Landell, Faganello, A. Schrank, Vainstein, M. Blackw. & P. Valente, 2008) (n=1) and *Trichosporon* spp. (n=1) against antifungals. Among the results, (54.3%) were classified as highly strong producers. *Trichosporon asahii* was the species that had the greatest number of isolates fit in this category (Lara et al., 2023).

We can observe that the species *T. asahii*, in addition to being the most isolated species, in the samples surveyed, this fungus is recognized worldwide as the emerging agent in fungal infections and receives more attention in cases of invasive and superficial Trichosporonosis, as highlighted in this study and which received greater agreement in the phylogenetic analyses. Since several species were recently proposed, by Chinese researchers (Liu et al., 2015a, b), they confirmed 22 and 16 species in these genera, *Apiotrichum*, *Trichosporon, Cutaneotrichosporon, Haglerozyma, Pascua, Prillinger, Vanrija,* within the family *Trichosporon*aceae (Table 5).

Several clinical studies involving microbiological evaluations have shown the existence and action of this fungal genus, in reports of infrequent species in the case of *T. japonicum* in patients with transplants and patients with leukemia (Ağirbasli et al., 2008; Albitar-Nehme et al., 2022; Hu et al., 2021) and more recent research reporting a range of species as human pathogens capable of causing invasive infections caused by *T. asahii* in patients with chronic renal failure and cystic fibrosis (Almeida Júnior and Hannequin, 2016), from blood isolates involving *A. mycotoxinivorans* (Dabas et al., 2017), *Cutaneotrichosporon debeurmannianum* isolates in blood and urine samples (Nath et al., 2018), *Apiotrichum veenhuisii* isolates in patients with myeloid leukemia (Lara et al., 2019) and hospital isolates involving *T. asahii, T. inkin* and *T. faecale* (Francisco et al., 2019).

And a group of *Trichosporon* species, performed in a phenotypic, genotypic and proteomic comparison carried out by Brazilian researchers, involving 59 species

Table 5. Revision of species of the genus Trichosporon recognized as human pathogens.

Basidiomycete (*Trichosporonales*, *Trichosporonaceae*) yeast species incriminated as pathogens of medical importance			
Lineage/Clade	**AUTHORS**		
	Colombo et al., (2011)	**Liu et al., (2015 a, b)**	**Takashima et al., (2019, 2020)**
Brassicae	*Trichosporon brassicae*	*Apiotrichum brassicae*	*Apiotrichum brassicae*
	T. domesticum	*A. domesticum*	*A. domesticum*
	T. montevideense	*A. montevideense*	*A. montevideense*
	T. scarabaerorum	*A. scarabaeorum*	*A. scarabaeorum*
Porosum	*T. lignicola*	*A. lignicola*	*A. lignicola*
	T. porosum	*A. porosum*	*A. porosum*
	T. sporotrichoides	*A. sporotrichoides*	*A. sporotrichoides*
	T. wieringae	*A. wieringae*	*A. wieringae*
Gracile	*T. gracile*	*A. gracile*	*A. gracile*
	T. akiyoshidainum	*A. akiyoshidainum*	*A. akiyoshidainum*
	T. cacaoliposimilis	*A. cacaoliposimilis*	*A. cacaolipasimilis*
	T. chiropterorum	*A. chiropterorum*	*A. chiropterorum*
	-	-	*A. coprophilum*
	T. dehoogii	*A. dehoogii*	*A. dehoogii*
	T. dulcitum	*A. dulcitum*	*A. dulcitum*
	T. laibachii	*A. laibachii*	*A. laibachii*
	T. loubieri	*A. loubieri*	*A. loubieri*
	T. mycotoxinivorans	*A. mycotoxinivorans*	*A. mycotoxinivorans*
	T. multisporum	*T. multisporum*	-
	T. otae	*A. otae*	*A. otae*
	T. siamense	*A. siamense*	*A. siamense*
	T. vadense	*A. vadense*	*A. vadense*
	T. veenhuisii	*A. veenhuisii*	*A. veenhuisii*
	T. xylopini	*A. xylopini*	*A. xylopni*
	T. gamsii	*A. gamsii*	*A. gamsii*
Cutaneum	*T. cutaneum*	*Cutaneotrichosporon cutaneum*	*Cutaneotrichosporon cutaneum*
	T. cavernicola	*C. cavernicola*	*C. cavernicola*
	T. debeurmannianum	*C. debeurmannianum*	*C. deberumannianum*
	T. dermatis	*C. dermatis*	*C. dermatis*
	T. guehoae	*C. guehoae*	*Pascua guehoae*
	T. jirovecii	*C. jirovecii*	*C. jirovecii*
	T. middelhovenii	*C. middelhovenii*	*C. middelhovenii*
	T. moniliforme	*C. moniliforme*	*C. moniliforme*
	T. mucoides	*C. mucoides*	*C. mucoides*
	T. oleaginosus	*C. oleaginosum*	*C. oleaginosum*
	T. smithiae	*C. smithiae*	*C. smithiae*
	T. terrícola	*C. terricola*	*C. terricola*
	-	*C. arboriformis*	*C. arboriformis*
	-	*C. curvatum*	*C. curvatum*
	-	*C. cyanovorans*	*C. cyanovorans*
	-	*C. daszewskae*	*C. daszewskae*
	-	*C. hagleorum*	*C. hagleorum*
	-	-	*C. spelunceum*
Trichosporon/Ovoides	*T. aquatile*	*Trichosporon aquatile*	*Trichosporon aquatile*
	T. asahii	*T. asahii*	*T. asahii*
	T. asteroides	*T. asteroides*	*T. asteroides*
	T. caseorum	*T. caseorum*	*T. caseorum*
	T. coremiiforme	*T. coremiiforme*	*T. coremiiforme*
	T. dohaense	*T. dohaense*	*T. dohaense*
	T. faecale	*T. faecale*	*T. faecale*
	T. inkin	*T. inkin*	*T. inkin*
	T. insectorum	*T. insectorum*	*T. insectorum*
	T. japonicum	*T. japonicum*	*T. japonicum*
	T. lactis	*T. lactis*	*T. lactis*
	T. ovoides	*T. ovoides*	*T. ovoides*
	T. shinodae	*T. shinodae*	*T. shinodae*
	T. chiarelli	*Haglerozyma chiarellii*	*Haglerozyma chiarellii*
***Tausonia* (*Guehomyces*)**	*T. pullulans*	*Tausonia pullulans*	*Tausonia pullulans*
Effuseotrichosporon	*T. vanderwaltii*	*Effuseotrichosporon vanderwaltii*	*Effuseotrichosporon vanderwaltii*
Vanrija	-	*Vanrija fragicola*	*Prillingera fragicola*
	-	*Vanrija humicola*	*Vanrija humicola*

Source: Colombo et al. (2011); Liu et al. (2015a, b); Takashima et al. (2020). Adapted Leite Júnior, D.P.

of the genus *Trichosporon*, isolating *T. ashahii* as the predominant species, followed by *Cutaneotrichosporon debeurmanniarum*, *C. dermatis, C. mucoides, Apiotrichum montevidennse, A. venhuisii, T. insectorum, T. japonicum, T. inkin, T. coremiiforme* and *T. faecale* (Lara et al., 2021).

In 2023, Lara and researchers (Lara et al., 2023) investigated these yeasts, verifying their ability to form a biofilm; against the antifungals fluconazole, itraconazole, amphotericin-B, voriconazole and caspofungin comparing the broth microdilution technique (EUCAST) with the commercial method E-test. It was observed that the ability to form biofilm on the surface of the polystyrene plates of the isolates was 54.3% of the samples and all were considered strong producers.

Of the species of this fungal genus, six were highlighted in the literature, and were associated with different types of human infections: *T. asahii* and *C. mucoides* involved in deep and severe infections, *C. cutaneum* and *T. asteroides* involved in superficial infections, and *T. ovoides* and *T. inkin* related to the involvement of capillary white piedra and the genital region (Sugita et al., 2000), involving the inguinocrural and perianal areas. Researchers from São Paulo/Brazil (Colombo et al., 2011) Trichosporonosis infections are associated with *T. asahii, C. mucoides* and *T. asteroides*, involved in fungemia, urinary tract infections, peritonitis and endocarditis and other inflammations; *C. cutaneum*, associated with pneumonitis and allergic hypersensitivity and *T. inkin, C. cutaneum, T. ovoides* and *A. loubieri*, involved in manifestations of white piedra and superficial infections.

According to reports by Rodriguez-Tudela (Rodriguez-Tudela et al., 2005), *T. asahii* seems to be the most frequent species in cases of systemic infection by *Trichosporon* species than in superficial infections. It should be taken into account that this species has a "cosmopolitan" aspect, as described by several authors (Francisco et al., 2019; Dabas et al., 2017; Sugita et al., 2000) and can often be isolated from different infectious sites, however, in this work, we can see that *T. asahii* was the predominant species when it comes to materials collected from superficial infections.

The frequency of dermatological manifestations in invasive or disseminated Trichosporonosis is considerable, as Liao and researchers (Liao et al., 2015) point out in an epidemiological study of 185 cases of fungemia by *Trichosporon*, in this result, 60% showed involvement of a single infected tissue, including the skin, which subsequently spread to other organs.

The genus *Trichosporon* is considered a primary pathogen; it is part of the microbiota of the skin surface and its annexes. Regarding nail infections involving this fungal genus, the literature has shown a higher prevalence in young adults, with a tendency in males, although genus is not an influencing factor for the presence of the disease (Robles-Tenorio and Tarango-Martinez, 2022; Leite Júnior et al., 2011; Colombo et al., 2011; Almeida Júnior and Hennequin, 2016; Cano-Pallares et al., 2016). Some researchers report that this type of infection is common in the pediatric group (Almeida Júnior and Hennequin, 2016; Roselino et al., 2008).

Mexican researchers found *C. cutaneum* in 42% of pediatric patients with onychomycosis and athlete's foot in a rural community in that country (Archer-Dubon et al., 2003). Garnica et al., (Garnica et al., 2018), in the same country, reported in a study that evaluated 1,408 patients diagnosed with onychomycosis, isolating the genus *Trichosporon* in 18 patients with a higher frequency for women.

In a survey by Indian researchers (Kotwal et al., 2018), three species of *Trichosporon*, *T. asahii, T. asteroides,* and *T. faecale* were isolated from the infected nails of three female members of a family. Among the species, *T. asahii* was recovered from the nail samples of the three members, thus confirming its recognition as the main pathogenic species of onychomycosis. In our series, the frequency of nail Trichosporonosis was small, with frequency (2; 8%) recorded in the male population, no cases were recorded in the female and pediatric population.

In another series, *T. ovoides* was obtained mainly as a single agent, from nail scrapings, but also found in mixed cultures associated with the yeast of the genus Candida, dermatophytes and other non-dermatophytic fungi (Magalhães et al., 2016).

Brazilian researchers, Silvestre-Jr (Silvestre Júnior et al., 2010) in their research involving hospitalized patients found *C. cutaneum* prevalent in Caucasian men, in the illiterate group, aged over 70 years. Mattede (Mattede et al., 2015) isolated the eukaryotic agent in urinary infections, having as a criterion suggestive of white piedra in the hair of the armpits and pubic region of their male patients.

In another study carried out in Brazil, by researchers from São Paulo, 24 urinary infections by *T. asahii*. Were observed in patients with renal failure, of which 71% were men, aged over 70 years (Fagundes Júnior et al., 2008).

Zhang and researcher (Zhang et al., 2011) reports that the opportunistic pathogen *T. asahii* is part of the cutaneous fungal microbiota in humans, and skin colonization in healthy individuals has been observed, with increased frequency in middle-aged men versus other age groups. Mattede (Mattede et al., 2015) confirms the infection in men over 70 years of age. Silvestre-Jr (Silvestre Júnior et al., 2010) reports that medically important *Trichosporon*, and other yeasts (Leite Júnior et al., 2011), is present in the perigenital region of normal male individuals and that the type of species present correlates with age.

In a Scandinavian study, from the 80's (Torssander et al., 1985) the high colonization of this yeast in homosexuals was characterized and the study related to the important factor for colonization in this population. However, several records in the literature (Garnica et al., 2018; Kotwal et al., 2018; Roselino et al., 2008) report marked differences in relation to colonization by *Trichosporon*, with a divergence in relation to sexual genus.

In our records we found a similarity in relation to the ethnicities of yeast infections of the genus *Trichosporon*, and the results found are consistent with other researchers; black men (13; 52%) were the most frequently infected, with the species *T. asahii* as predominant; followed by brown and caucasian men, other ethnicities were not found. When we reported the anatomical sites, it was observed that the genito-crural region (13; 52%) was the most identified with this type of infection, followed by the genital region (Table 3).

Previous studies reported by Kalter (Kalter et al., 1986) investigated in 166 young men with genital complaints, found 40% of infections in scrotal hair, finding a predominance in black men (54%) than in other ethnic origins (46%). Elner (Ellner et al., 1991) also found a higher prevalence in black men, in inguinal skin scrapings from 322 asymptomatic volunteers. According to Jenks (Jenks et al., 2023) emphasizes that black and African American individuals appear to be at high risk for superficial and invasive infections, which may be related to underlying social determinants of health, disparities in access to health care and other socioeconomic disparities.

Records in 2002 report that in Brazil, *T. inkin* and *T. asahii* were reported colonizing the hair shaft in the anal and perianal regions of HIV-positive patients (Pontes et al., 2002). In collaboration, Spanish and Brazilian researchers (Ribeiro et al., 2008) performed isolates from Brazilian patients, analyzing sequencing of the IGS1 region and revealed that different species of *Trichosporon* spp. were grouped into distinct clades and in the samples from superficial regions, *T. asahii* predominated (43%), followed by *T. faecale* (24%) and *T. inkin* (14%) and in smaller percentages *T. coremiforme, T. japonicum* and *T. asteroides*.

However, in 2010, Brazilian researchers (Silvestre Júnior et al., 2010) found *T. asahii* less frequently in their series of superficial scrotal, perianal, and inguinal samples from the body and urine of male hospitalized patients. The following species identified *C. cutaneum* (46%), *T. asteroides* (53%), *T. ovoides* (17%), *T. inkin* (71%), *C. mucoides* (92%) and *T. asahii* (25%). In 2016, Chinese researchers reported a case of pubic hair infection by *T. inkin* in a 36-year-old male (Zhuang et al., 2016).

Most cases of genital white piedra and infections by *Trichosporon*, second in incidence, involve men between 15 and 44 years of age, according to the records found by Kalter (Kalter et al., 1986), despite reports from other researchers (Mattede et al., 2015; Silvestre Júnior et al., 2010; Fagundes Júnior et al., 2008) in their series reported that men aged >70 years are also more affected.

A study carried out on white piedra carried out in the State of Paraíba/Brazil, the researchers reported that infections by the genus *Trichosporon* affect patients of both sexes, with most cases involving women and children (Pontes et al., 2002). This statement is related to the results found by Roselino (Roselino et al., 2008) and Diniz & Filho (Diniz and Filho, 2005), who indicate that females and girls are the groups most affected by white piedra, in studies carried out in Brazilian cities, São Paulo and Espirito Santo, respectively.

In our series, genital Trichosporonosis is quite common in young men in the second to fourth decades of life. The results found in this series show that men between 21 and 30 years of age were the most affected by this type of infection. *T. asahii* was also the predominant species (Table 3). The predisposing factors for this type of infection are in line with results previously recorded with other population groups and may be associated with the hot and humid climate of the central west region of Brazil (Leite Júnior et al., 2014), aided by the habit of using tight clothes; the production of excessive sweating, daily stress and even associated with hygiene conditions, often precarious and cultural habits distinct from the region, can influence the determining factors for the installation of the infection in the researched population (Leite Júnior et al., 2011).

In this sense, Brazilian researchers (Pontes et al., 2002) reported in their series carried out in the northeast region of Brazil, that the predisposing factors for the acquisition of infections; are related to the low socio-economic level; the poor notion of hygiene and by people who live together, thus influencing the transmission of this mycosis. In addition, Indian researchers reported in their research that the predisposing factors for fungal acquisition are low socioeconomic status, lack of hygiene, exposure to water and dirt, and climatic conditions, observed for nail infection in these patients from that country (Kotwal et al., 2018).

Reports by Bhalani (Bhalani et al., 2008) indicate that the use of men's undergarments can greatly contribute to the permanence of dermatoses and other infections. Indicating that the incidence of infections is associated, when there is contact of the intimate part when passing through the feet and with the inguinal region and consequently reaching the genital organ. Mycotic infections of the perineum can commonly present in patients with previous mycotic infections of other regions, including feet and hair (Duldulao et al., 2019; Leite Júnior et al., 2014; Leite Júnior et al., 2011).

An extensive work carried out by researchers in Gabon/Africa involved research on white piedra infections in the population of that country in 1994. The first work carried out an analysis of 449 female superficial inguinal specimens, having the species *T. inkin, C. mucoides, T. asahii*. In the second study, 81 of the 449 Gabonese patients examined were positive for genito-pubic white piedra. Three species have been recognized *C. mucoides* (25), *T. inkin* (20) and *T. asahii* (7) (Thérizol-Ferly et al., 1994a; Therizol-Ferly et al., 1994b). Another case of genital white piedra was isolated in a 32-year-old female patient in Spain, with the isolated etiologic agent being *T. inkin* (Andreu et al., 2009).

Regarding *Trichosporon* infections on the scalp, in Brazil, 10 records of clinical samples in children under 10 years of age; obtained *T. ovoides* (7) as predominant and *T. inkin* (2) isolated, having white piedra in the scalp as agents (Magalhães et al., 2008). A study in Brazil included the isolation of *T. inkin* in three members of the same family, mother, daughter and son, causing capillary Trichosporonosis (Richini-Pereira et al., 2012) Brazilian researchers reported another record of Mother and daughter presenting the same infection, with tiny nodules (1 to 2 mm), yellowish-white on the hair shaft, with *T. ovoides* as the etiological agent (Ferreira et al., 2019).

In India, a case of group infection was recorded in a mother and daughter in India, due to *T. asahii* infection (Roshan et al., 2009). Another research in India reported a case of white piedra of the scalp in a 32-year-old woman caused by *T. ovoides*, diagnosed by clinical, trichoscopic, microbiological and molecular methods (Gaurav et al., 2022).

Most cases occur in tropical and subtropical countries. A systematic review of 106 cases on the American continent showed that 75.58% of cases of white piedra originated in South America, predominantly in Brazil (76%) and Colombia (12.6%). In North America (25.48%), Mexico (63%) was responsible for most cases. Of this amount, 89 (84%) of the patients were under 15 years old and more than 90% of the cases of capillary piedra were female patients (Ramirez-Soto et al., 2019).

In another study, involving capillary Trichosporonosis, in Mexican patients, 14 cases of white piedra, all located on the scalp and 1 case involving affected scalp and scrotal hair, of these 9 cases occurred in children < 15 years and most case records were female, recording *T. ovoides, T. inkin* and *C. cutaneum* (Bonifaz et al., 2019).

Trichosporon spp. is emerging as opportunistic agents that cause systemic disease in immunocompromised hosts. Species considered rare in infection are being described in the literature. *Gueromyces pullulans* (=*Tausonia pullulans*), was isolated from air samples and nowadays, Moylett and researchers (Moylett et al., 2003) reported cases of *T. pullulans* infection in patients with granulomatous diseases and reviewed five additional cases from the literature.

The scientific literature shows cases involving the three species identified in this study: *T. asahii, T. inkin* and *T. faecale*. *Trichosporon asahii* is arguably the most prevalent species in human anatomical sites, as reported by Brazilian researchers, 98% in urinary infections and 79% in blood infections and 36% in anal swabs and 33% in skin, hair and nails, followed by the species *T. inkin* and *T. faecale* (Francisco et al., 2019). Indian researchers (Dabas et al., 2017) found *T. asahii* (80.9%) as the predominant species in blood infections, followed by *T. faecale*. These reports demonstrate the high variability and plasticity of these eukaryotic organisms in adapting to human infection conditions. In our series, we found that the results found for *T. asahii, T. inkin* and *T. faecale,* are consistent with the records of the aforementioned researchers, for skin, hair and nail conditions.

T. inkin is the etiologic agent of white piedra, a benign disease of the hair shafts. Invasive fungal infection caused by this species has been described in patients with predisposing factors, such as oncohematological diseases and neutropenia (Colombo et al., 2011). Recently, invasive fungal infection caused by *T. inkin* has been reported in patients undergoing solid organ transplantation (Almeida Júnior and Hennequin, 2016).

Historically, given the distributions of species of *Trichosporon* spp., reported by researchers around the world; the species *T. faecale* has emerged as an opportunistic human pathogen in recent years, with its first record being a report of tinea pedis infection in a patient in Germany in 2008 (Hahner et al., 2008), another case involving foot infection in Iran. In 2012 (Fallahi et al., 2012), with the first episodes of fungemia recorded from 2009 onwards, were described by Taj-Aldeen (Taj-Aldeen et al., 2009), then as the causative agent of an invasive Trichosporonosis with fungemia and skin lesions in a patient with hematological disease (Pérard et al., 2015) and then in 2017, in the case of Trichosporonosis in the report by Dabas (Dabas et al., 2017).

Finally, dermatophytosis is superficial mycosis caused by filamentous, hyaline and septate fungi called dermatophytes. As dermatophytosis are contagious infections with high prevalence and characterized as zoonosis, it is not uncommon to see humans affected by this disease. They are commonly observed in tropical and temperate climates, particularly in countries with areas of hot and humid climatic conditions, as are the climatic characteristics of the region under study. In seven patients, agents of dermatophytosis were found in the studied population. The most frequently isolated genus was *T. rubrum*, followed by *M. canis* and *E. floccosum*, all isolated from the skin of the genital and inguinal-crural region.

Trichosporon can be misdiagnosed as contaminants, or simply skin microbiota, resulting in attributing the infection to dermatophytes, when in fact *Trichosporon* can be the etiological agent in 10% to 40% of superficial infections, depending on the geographic area and population studied (Ghahri, 2021) and being associated with dermatophytes, mainly *T. rubrum*, frequently for the male population (Carrascal-Correa et al., 2020; Han et al., 2000; Leite Júnior et al., 2014; Thérizol-Ferly et al., 1994a).

Representing the first or second most common non-Candida yeast involved in life-threatening fungal infections (Chen et al., 2021; Almeida Júnior and Hennequin, 2016; Colombo et al., 2011); the prevalence of non-*T. asahii* species shows geographic differences and distinct characteristics, as well as *T. inkin* and *T. faecale* being frequently reported, although in this study *T. asahii* was the most commonly isolated species in superficial infections, other researchers report that *T. inkin* is the most prevalent agent in superficial infections (Almeida Júnior et al., 2021; Guo et al., 2019; Francisco et al., 2019; Liu et al., 2015a).

In this study, two isolates of yeasts of the genus Candida were found: a skin sample in the scrotal region isolated from *Candida albicans* and another skin sample from the inguinal region, isolating *C. parapsilosis* associated with *T. inkin*. Some researchers in their series found *C. parapsilosis*, a yeast-like fungal species implicated in white piedra, synergistically with *Trichosporon* and as the only pathogen (Soumare et al., 2022; Sharma et al., 2019; Taj-Aldeen et al., 2004).

Associations of other yeasts with the *Trichosporon* agent have been demonstrated in recent research that *C. albicans* and *Cryptococcus neoformans*, as the first or second most common yeast, causing an increase in the pathogenicity and evasion of the immune response, involved in life-threatening fungal infections (Chen et al., 2021; Almeida Júnior and Hennequin, 2016; Colombo et al., 2011). Mexican authors, in 2022, report that *Trichosporon* infection is considered the second most common opportunistic yeast infection in patients with immunosuppression and that the pathogen can live as a commensal on the skin and cause disease in immunocompetent individuals (Taj-Aldeen et al., 2004).

The prevalence of non-*T. asahii* species have geographic differences and distinct characteristics, as well as *T. inkin* and *T. faecale* being frequently reported. In this study, *T. asahii* was the species most commonly isolated in superficial infections, as reported by other researchers in their series of prevalence in superficial infections (Francisco et al., 2019; Liu et al., 2015a; Guo et al., 2011; Han et al., 2000)

Considered the classic agent of mycosis, *Trichosporon* species are currently recognized as emerging opportunistic pathogens (Almeida Júnior et al., 2021). In view of the facts, the clinical presentation of *Trichosporon* infections varies according to the anatomical site affected. The successive isolation of *Trichosporon*, observed in this study, in the microbiota of the skin and mainly in the inguinal-crural region in a male population, characterizes this fungal genus and is considered a primary pathogen of superficial infections.

4.1. Climate and Trichosporon

As for the findings related to climatic conditions and yeasts of the genus *Trichosporon*. These findings allow us to infer that the high temperatures, which occur on average from July to September, considered the dry period of drought in Central Brazil, contributed to a greater manifestation of fungal infections. The production of body sweating, associated with the characteristics of each individual, lifestyle, professional profile and personal hygiene; associated with these factors probably facilitated the fixation and dispersion of this infectious agent in the studied population.

Ruan (Ruan et al., 2009) found associations between this infectious agent and patients undergoing treatment in China, and identified that the incidence of trichosporonosis peaked in May, in the period from 2000 to 2008, at the beginning of summer in northern Taiwan. Regional researchers, in other cases, defined that the use of thick fabric clothes, which cause intense perspiration, were factors considered for the permanence and fixation of fungal infections (Leite Júnior et al., 2011) and also defined the anatomical sites inguinocrural and in the feet, as the regions most affected by these infections, when related to climatic conditions, hot and humid, predisposing to infection by fungal entities (Leite Júnior et al., 2014) Several reports claim that patients with crural or inguinal infections often have concomitant superficial infections in the feet, associated with sweating, heat and climatic conditions (Robles-Tenorio and Tarango-Martinez, 2022; Lara et al., 2021; Lara et al., 2019; Ebrahimi et al., 2019; Heidrich et al., 2015; Leite Júnior et al., 2014; Leite Júnior et al., 2011; Colombo et al., 2011; Ruan et al., 2009; Bhalani et al., 2008; Thérizol-Ferly et al., 1994b, Ellner et al., 1991).

Currently, high temperatures, related to global warming, appear to be a triggering factor for changes in fungal species, which presumably, according to some scientific reports, these changes are promoting greater fungal resistance and greater potential for these entities to cause disease (Gusa et al., 2023), as described by research involving *Candida auris* (Satoh & Makimura, 2009), on the adaptation of this fungus to climate change (Casadevall et al., 2019), which revealed this fungal species possibly facilitated by climate change; also *Aspergillus fumigatus* (Fresen., 1863) resistant to azoles, resistant to the use of azole fungicides in agricultural environments; and *Trichophyton indotineae* (R. Kano, U. Kimura, M. Kakurai, J. Hiruma, H. Kamata, Y. Suga & K. Harada, 2020 in [Kano R et al. (2020)], due to the use of antifungal creams containing corticosteroids sold without a prescription (Lockhart et al., 2023). Links to a warmer planet could mean that fungal infections are increasing at a faster rate than the development of treatments for fungal diseases. Surveillance is critical to monitor the emergence of fungi as well as the rise in global antifungal resistance.

5. Conclusion

We are only beginning to understand how the interconnections between people, animals, and the environment affect fungal diseases. In conclusion, based on the great variability of natural habitats in which the species already described are related to the human microbiota, anatomical sites, and a range of different substrates and diverse reservoirs, *Trichosporon* species may play an important role in human infections, together with other fungal genera that have been standing out in view of the clinical picture presented by the genus.

It should be noted that the hot and humid bioclimatic conditions that exist in Cuiabá, Mato Grosso, can provide an extremely favorable environment for the development of fungal infections, a factor that is enhanced when associated with the extreme conditions of each individual.

This study demonstrated that the three species of *Trichosporon* isolated in this study are important agents of skin infections, are present in the anatomical sites of normal individuals and it is important to know from an epidemiological and clinical point of view to define new methodologies for the treatment of these emerging and reemerging species. Often, the monitoring of outbreaks and infections caused by these microorganisms can cause, in addition to superficial infections, invasive infections, which in recent years have also shown to be quite worrying for medicine. Immediate diagnosis and management inherent to this infectious agent are essential for the treatment and resistance to antifungals available for the treatment of serious infections.

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